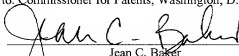


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Date of Signature and Deposit: September 27, 2001



Jean C. Baker

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Rui Sousa, et al. Date: September 27, 2001

Serial No.: -- Group Art Unit: --

Filed: Herewith Examiner: --

Title: METHODS FOR USING MUTANT RNA POLYMERASES WITH REDUCED DISCRIMINATION BETWEEN CANONICAL AND CANONICAL NUCLEOSIDE TRIPHOSPHATES File No.: 310307.90061

PRELIMINARY AMENDMENT

Commissioner For Patents
Washington DC 20231

Dear Sir:

In conjunction with filing the enclosed continuation application, please amend the application as follows:

In the Specification:

On Page 1, below the title and above the subtitle "Field of Invention," please insert the following subsection:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of 09/643,189, filed on August 21, 2000, which is a continuation of 09/100,803, filed on June 19, 1998, U.S. Patent No. 6,107,037, which is a division of 08/713,331, filed on September 13, 1996, U.S. Patent No. 5,849,546.

Please delete Pages 59 and 60 and insert the enclosed paper copy of the sequence listing to the end of the application (after the ABSTRACT).

In the Claims:

Please cancel Claims 1-40 and add new Claims 41-87 as follows:

41. A method for determining sequence of a nucleic acid molecule, comprising the steps of:

- a) synthesizing a nucleic acid molecule de novo from a RNAP promoter sequence in a reaction mixture containing a mutant T7-type RNA polymerase in each of four separate reactions, wherein said mutant T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydroxy or a hydrogen or a fluorine at the 2'-position, and further comprising a ddNTP, such that each of the four separate reactions forms a plurality of reaction products of differing length, the length of said reaction products indicating the positions or the type of base corresponding to the incorporated ddNTP, and
- b) evaluating the reaction products so that the sequence of the template molecule may be deduced.

42. The method of Claim 41, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

43. The method of Claim 41, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP.

44. The method of Claim 41, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, and 5-hydroxy-methyl-dCTP.

45. The method of Claim 41, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 2'-deaza-2'-F-GTP, 2'-F-ITP, 5-methyl-2'-F-CTP, and 5-hydroxymethyl-2'-F-CTP.

46. A method for determining the sequence of a nucleic acid molecule, comprising the steps of:

a) synthesizing a nucleic acid molecule by extending a primer, wherein at least part of the primer is complementary to a template molecule so as to anneal therewith, in a reaction mixture containing a mutant T7-type RNA polymerase in each of four separate reactions, wherein said mutant T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, each comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydroxy or a hydrogen or a fluorine at the 2'-position, and further comprising a ddNTP, such that each of the four separate reactions forms a plurality of reaction products of differing length, the length of said reaction products indicating the positions of the type of base corresponding to the incorporated ddNTP; and

b) evaluating the reaction products so that the sequence of the template molecule may be deduced.

47. The method of Claim 46, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

48. The method of Claim 46, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP.

49. The method of Claim 46, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, and 5-hydroxymethyl-dCTP.

50. The method of Claim 46, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 2'-deaza-2'-F-GTP, 2'-F-ITP, 5-methyl-2'-F-CTP, and 5-hydroxymethyl-2'-F-CTP.